

Amendments to the Specification

Please replace paragraphs [0070], [0071], [0072], [0074], and [0075] with the following amended paragraphs:

[0070] Mouse whole kidney tissues (or cultured human proximal tubule cells, see below) were disrupted with a Tissue Tearor™ (Biospec Products, Racine, WI). Total RNA from control and ischemic kidneys was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA), and quantitated by spectrophotometry.

[0071] Detailed descriptions of microarray hardware and procedures have been previously published (3). Briefly, for each experiment, 100 µg of purified total mouse kidney RNA was reverse transcribed with Superscript II® reverse transcriptase (Life Technologies, Rockville, MD) in the presence of Cy3-dUTP (Amersham, Piscataway, NJ) for controls and Cy5-dUTP for ischemic samples. The cDNA samples were purified using a Microcon® YM-50 filter (Millipore, Madison, WI), and hybridized to microarray slides containing 8,979 unique sequence-verified mouse probes (3). Three separate animals were examined for each of the reflow periods, and at least two independent microarray experiments were performed for each of the animals. The array slides were scanned using a microarray scanner (GenePix® 4000B, Axon Instruments, Foster City, CA) to obtain separate TIFF images for Cy3 and Cy5 fluorescence. The signal intensities for Cy3 and Cy5 were determined for individual genes using the GenePix® Pro 3.0 data extraction software (Axon Instruments). Quality control and data analysis was completed as previously described (3).

[0072] An equal amount (1 µg) of total RNA from control and experimental mouse kidneys was reverse transcribed with Superscript II® reverse transcriptase (Life Technologies) in the presence of random hexamers according to the manufacturer's instructions. PCR was accomplished using a kit (Roche, Indianapolis, IN) and the following primers:

Mouse NGAL sense 5'-CACCACGGACTACAACCAGTTCGC-3' ;

Mouse NGAL antisense 5'-TCAGTTGTCAATGCATTGGTCGGTG-3' ;

Human NGAL sense 5'-TCAGCCGTCGATACACTGGTC-3'; and

Human NGAL antisense 5'-CCTCGTCCGAGTGGTGAGCAC-3'.

[0074] Frozen sections were permeabilized with 0.2% Triton™ X-100 in PBS for 10 min, blocked with goat serum for 1 hr, and incubated with primary antibody to NGAL (1:500 dilution) for 1 hr. Slides were then exposed for 30 min in the dark to secondary antibodies conjugated with Cy5 (Amersham, Arlington Heights, IL), and visualized with a fluorescent microscope (Zeiss Axiophot) equipped with rhodamine filters.

[0075] For co-localization of NGAL with Rab11, serial sections were first incubated with NGAL antibody or a monoclonal antibody to Rab11 (1:500 dilution; Transduction Laboratories), then with secondary antibodies conjugated with either Cy5 (for NGAL) or Cy3 (for Rab11) and visualized with rhodamine or fluorescein filters, respectively. For co-localization of NGAL with proliferating cell nuclear antigen (PCNA), sections were co-incubated with NGAL antibody and a monoclonal antibody to PCNA (1:500 dilution; Upstate), and was detection accomplished by immunoperoxidase staining (ImmunoCruz™ Staining System, Santa Cruz Biotechnology). For the TUNEL assay, we used the ApoAlert® DNA Fragmentation Assay Kit (Clontech). Paraffin sections were deparaffinized through xylene and descending grades of ethanol, fixed with 4% formaldehyde/PBS for 30 min at 4°C, permeabilized with proteinase K at room temperature for 15 min and 0.2% ~~triton~~ Triton® X-100/PBS for 15 min at 4°C, and incubated with a mixture of nucleotides and TdT enzyme for 60 min at 37°C. The reaction was terminated with 2X SSC, and the sections washed with PBS and mounted with Crystal/mount (Biomed, Foster City, CA). TUNEL-positive apoptotic nuclei were detected by visualization with a fluorescence microscope.